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The Effect of Salinity and Temperature on Spawning and Fertilization in the Zebra Mussel *Dreissena polymorpha* (Pallas) from North America

PETER P. FONG[1], KEIICHIRO KYOZUKA[2], JILL DUNCAN, STACY RYNKOWSKI, DANIEL MEKASHA, AND JEFFREY L. RAM*

Department of Physiology, Wayne State University, Detroit, Michigan 48201

Abstract. Zebra mussels have dispersed from their original site of introduction in the Great Lakes into the Mississippi River, Hudson River, and other watersheds in which they will encroach upon brackish water estuaries. To investigate their likelihood of reproductive success in such estuaries, we investigated the conditions of temperature, salinity, and acclimation under which spawning and fertilization could occur. Reproductive function of mussels that were acclimated to salinities up to 7.0 parts per thousand (ppt) at 12°, 20°, and 27°C for 1 to 21 days was tested. Reproductive function of non-acclimated mussels that had been maintained in fresh-water aquaria was also tested in various salinities. Spawning was induced by serotonin, previously demonstrated to elicit spawning of fertile gametes in fresh water. Successful fertilization was indicated by oocyte cleavage after adding sperm. Non-acclimated mussels spawned in salinities of 1.75 and 3.5 ppt at 12°, 20°, and 27°C, but not at 7.0 ppt. Fertilization using gametes from non-acclimated mussels occurred only in fresh water and at 1.75 ppt. Acclimation for as little as 2 days enhanced spawning. Fertilization rate in a salinity of 3.5 ppt improved within 4 days of acclimation and continued at a high level for as long as 21 days of acclimation. Although animals acclimated for 4 days in 3.5 ppt spawned readily when tested in salinities as high as 7.0 ppt, almost no fertilization occurred in 7.0 ppt. The reduction in fertilization at increasing salinities may be due in part to reduced sperm motility. Unfertilized oocytes remain intact for hours in fresh water; however, in salinities as low as 0.7 ppt, unfertilized oocytes tended to rupture within 2 hours. These data show that although sudden increases in salinity produce an immediate decrease in the reproductive capacity of zebra mussels, acclimation to brackish water can occur, and zebra mussels may be able to reproduce in brackish water below 7.0 ppt.

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*To whom all correspondence should be sent.

Present address: [1]Department of Biology, Gettysburg College, Gettysburg, PA 17325. [2]Asamushi Marine Biological Station, Tohoku University, Asmushi, Aomori, 039-34, Japan.

Introduction

Zebra mussels (*Dreissena polymorpha*) have spread rapidly throughout North America since their accidental introduction in the mid-1980s (Hebert *et al.*, 1989). Their geographical distribution and the factors controlling it have been the focus of a number of studies (Mackie *et al.*, 1989; Griffiths *et al.*, 1991; Strayer, 1991; Ramcharan *et al.*, 1992). Strayer and Smith (1993) predicted that the downstream movement of zebra mussels will eventually carry them to estuarine sections of North America. At present, zebra mussels occupy brackish waters in the Hudson River estuary at West Haverstraw, New York (Walton, 1992; D. L. Strayer, pers. comm.), and with populations already well established in the Susquehanna and Mississippi Rivers, areas such as Northern Chesapeake Bay and the Mississippi River delta are at particular risk of invasion.

Unlike most freshwater bivalves, zebra mussels are free-spawners, releasing large numbers of gametes directly into the water, where fertilization occurs (Sprung, 1987). However, environmental tolerance to salinity may not be the same for gametes as for adults. Thus, even though adult zebra mussels may be capable of withstanding a range of salinities, these conditions may be suboptimal or lethal for freshly released gametes and may interfere

with fertilization. Similar situations are common in brackish-water organisms. In the viviparous polychaete *Neanthes limnicola,* higher salinities reduce the number of juveniles born and probably interfere with oogenesis (Fong and Pearse, 1992). In the brackish-water macrophyte *Myriophyllum crispatum,* higher salinities block both sexual and asexual reproduction (James and Hart, 1993). Higher salinities reduce reproductive capacity in brackish-water populations of *Daphnia magna* (Arner and Koivisto, 1993). Eggs of the yellow perch (*Perca flavescens*) suffer higher mortality with increasing salinity (Victoria *et al.,* 1992). In the striped mullet (*Mugil cephalus*), lower salinity reduces fertilization and sperm motility (Lee *et al.,* 1992). Thus salinity may have salient effects on reproductive processes, including spawning and fertilization, in zebra mussels.

Estuarine and brackish-water populations of adult zebra mussels occur throughout Europe in both tidal and nontidal bodies of water (Ludyanskiy *et al.,* 1993); however, no experiments to date have examined the reproductive potential of zebra mussels in different salinities. If reproduction is reduced or inhibited in estuarine areas, then maintenance of adult populations in these areas will depend largely upon larvae derived from upstream sources, and local recruitment will be negligible. Such information is directly relevant for modeling zebra mussel spread (*e.g.,* Neary and Leach, 1992; Ramcharan *et al.,* 1992) in all North American estuaries at risk of invasion, as well as some brackish inland lakes. In the present paper, we report on the effects of salinity and temperature on spawning and fertilization in salinity-acclimated and non-acclimated zebra mussels.

Materials and Methods

Acclimation and spawning experiments

Animals (13–25 mm in length) were collected on several occasions in 1994, from late May to the end of June, from western Lake Erie at Monroe, Michigan, (41° 54′N, 83° 23′W). Mussels were immediately transported to the laboratory and maintained in a thermostatically controlled 70-gallon aquarium at 12°C until use. This main holding tank was originally filled with Detroit tap water, and at intervals the water was partially replaced (up to 20% per week) from the same source. As a result of feeding, sediments associated with animals, the animals themselves, etc., average ion concentrations in the aquarium water are somewhat higher than those in the tap water. This water has been measured to contain 1.0 m*M* sodium, 0.25 m*M* potassium, and 1.4 m*M* calcium (Walker and Ram, 1994).

For acclimation to different conditions of temperature and salinity, clusters of animals were transferred to separate aquaria with the requisite conditions. Before being used in an experiment, animals were individually transferred to vials having the temperature/salinity combination being tested, and except for acute tests, were maintained in individual vials under these conditions for 1 day before testing. A schematic diagram of a typical sequence of holding, acclimating, and testing conditions is illustrated in Figure 1.

Specifically, about five clusters (8 cm diameter) of mussels were placed into 2.5-gallon aquaria equipped with aquarium heaters and cascading water pumps for circulation. Groups of animals were acclimated to 12 different temperature-salinity combinations: aquaria were maintained at 12°, 20°, or 27°C, and at each temperature separate aquaria contained either aquarium water (AW, water from our main holding tank), or water of a higher salinity (1.75, 3.5, or 7.0 ppt). Desired salinities were achieved by diluting Instant Ocean with AW.

Animals were acclimated for up to 21 days without added food, and the water was changed every 3 days. Food was withheld so as not to introduce additional variables in water quality. In histological studies of *Dreissena* starved for 30 days, Bielefeld (1991) reported that gonads were relatively resistant to degeneration compared to digestive gland and that the gonad/body quotient decreased only slightly. Furthermore, as reported in this paper for controls (*e.g.,* AW, 20°C), spawning and fertilization continued to occur at high levels even after animals were maintained without food for up to 21 days.

In our first experimental series, spawning tests (described below) were conducted on days 0 (no acclimation), 1, 2, 6, and 13. Zebra mussels did not survive at 7.0 ppt for more than 2 days. Thus, after 2 days of acclimation, mussels to be tested for spawning in 7.0 ppt were taken from the 3.5-ppt aquaria and transferred to individual vials containing water at 7.0 ppt for 24 h before spawning was tested.

Spawning experiments on acclimated animals were repeated with a second group of animals, but in this case, long-term acclimation to salinities greater than 3.5 ppt was preceded by exposure to intermediate salinities, as described below. Animals tested for spawning on days 0 (no acclimation) and 1 were acclimated in AW, 1.75, 3.5, and 7.0 ppt at 12°, 20°, and 27°C. On day 4, mussels from AW, 1.75, and 3.5 ppt were tested along with mussels tested in 6.0 ppt that had been acclimated for 3 days in 3.5 ppt and then transferred to vials containing 6.0 ppt for the final 24 h preceding testing. On day 7, animals previously acclimated in 1.75 ppt were transferred to 3.5 ppt and those previously in 3.5 ppt were transferred to 5.0 ppt for an additional 7 days. On the 14th day of acclimation, some animals in 5.0 ppt were transferred to vials containing 6.0 and 7.0 ppt and were tested for spawning on the following day.

Spawning was tested by exposing animals to 10^{-3} *M* serotonin (5-hydroxytryptamine; 5-HT), which, as shown

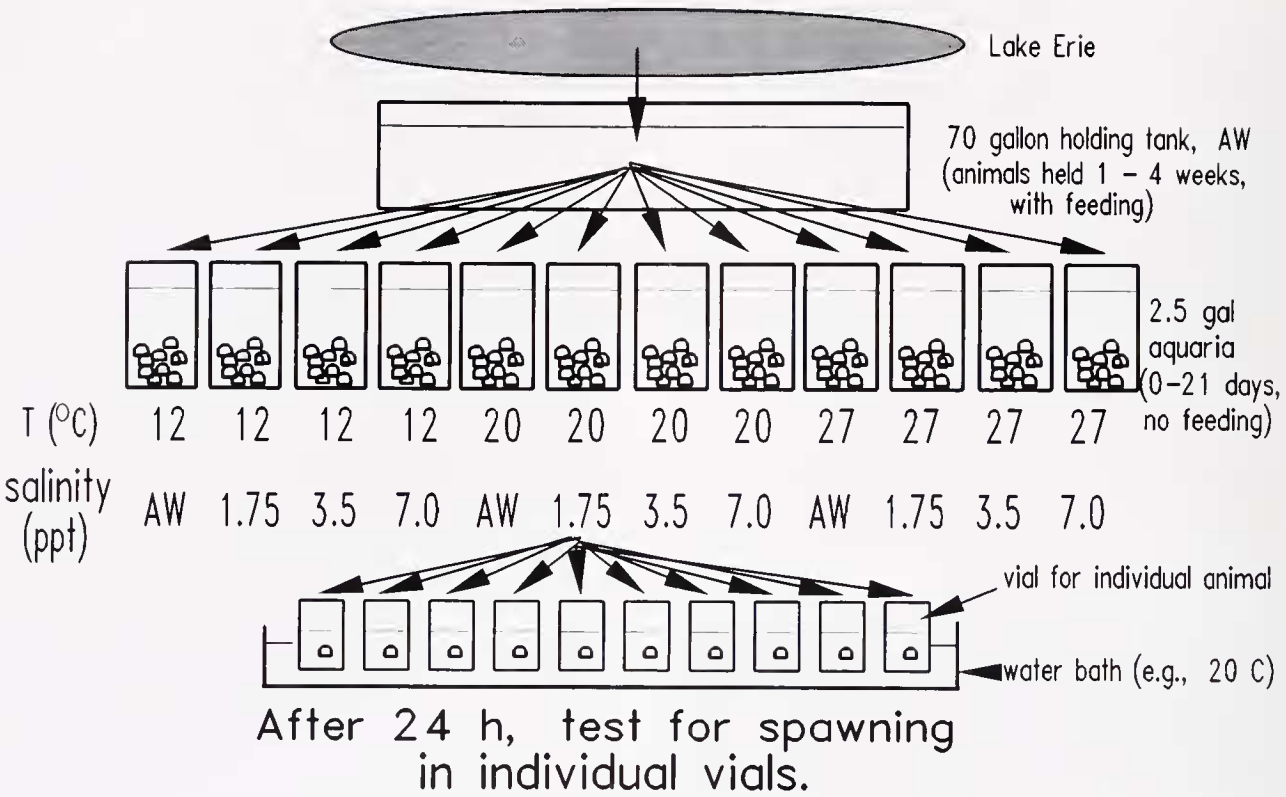

Figure 1. Diagram of the experimental design for testing spawning responsiveness of zebra mussels acclimated to various conditions of salinity and temperature. Testing of spawning responses of individual animals, illustrated here for 10 animals from just one temperature/salinity condition, was similarly performed for all other conditions. Non-acclimated animals (0 days in the 2.5-gal aquaria) went directly from the 70-gal holding tank of aquarium water (AW) into individual vials for testing with serotonin after 1 h in their individual temperature/salinity conditions.

by Ram *et al.* (1993), reliably elicits spawning in fresh water. About 24 h prior to the spawning test, acclimated animals were transferred to vials (1 mussel/vial, 10–15 animals per salinity/temperature condition) containing 9 ml of water at the same salinity and temperature in which they had previously been acclimated. Temperature was maintained by immersing the vials in thermostatically controlled water baths. The test was begun by the addition of 1 ml of 10^{-2} *M* 5-HT to each vial (for a final concentration of 10^{-3} *M*), and spawning was assessed visually and microscopically for up to 4 h after 5-HT addition. After 4 h, the gonads of all nonspawners were dissected and their sex and reproductive maturity determined under compound microscopy to calculate percent spawning in each group. Non-acclimated animals were tested with identical procedures except that they were transferred from the main holding aquarium (AW, 12°C) to vials at the appropriate salinity and temperature and tested for spawning by adding 5-HT 1 h after transfer.

To investigate whether salinity had an effect on the motility of freshly released sperm, we exposed sperm from non-acclimated mussels spawned in AW to salinities of 1.75, 3.5, and 7.0 ppt at room temperature (23°C). Sperm from salinity-acclimated mussels were similarly observed in their respective spawning salinities. Sperm motility was assessed by visual observation with a compound microscope (400×). Sperm were classified as either highly motile (the usual state of sperm in AW), reduced in motility, or immotile.

Fertilization experiments

We tested the effect of salinity on fertilization of oocytes obtained from salinity-acclimated and non-acclimated zebra mussels. Acclimated (for 4 days and 21 days at 20°C) and non-acclimated mussels (n = 30 mussels in each salinity) were placed in individual 20-ml vials in 9 ml of water having salinities of AW, 1.75, 3.5, and 7.0 ppt. All animals tested in 7.0 ppt were initially acclimated in 3.5 ppt and then transferred to 7.0 ppt overnight at 20°C before spawning was induced. To each vial, 1 ml of 10^{-2} *M* 5-HT was added until spawning occurred.

Sperm suspensions were made by mixing equal volumes of sperm from 3 to 5 males at each salinity into a separate vial. These sperm suspensions were then diluted to a density of 10^{10} sperm/ml measured with a hemacytometer. A volume of 10 μl of diluted sperm suspension was added to wells of a 24-well culture plate containing 1.0 ml of oocyte suspension (see below) for a final concentration of 10^8 sperm/ml. At this sperm concentration, high fertilization rates (>75%) can be obtained (data in this paper and unpublished data by K. Kyozuka in this laboratory), although polyspermy may also be occurring (Misamore *et al.*, 1994).

Egg suspensions from single females were prepared by counting the number of spawned oocytes in a 20-μl pipette and adjusting the volume to get a concentration of 1000 oocytes/ml. Oocytes were then diluted by 50% for a final concentration of 500 oocytes per well in 1.01 ml of sperm-egg water, or 1.0 ml of egg suspension alone (no-sperm controls in the 21-day acclimation experiment). All fertilization tests were done at room temperature (23°C). Oocytes were observed at 200× on an inverted microscope up to 3.5 h after the addition of sperm. Between 67 and 124 oocytes from each well were assessed for fertilization, as indicated by cleavage to at least the two-cell stage within 3.5 h. For mussels acclimated for 21 days, we also enumerated the number of oocytes that had ruptured.

Statistical analysis

Effects of acclimation to various salinity and temperature combinations were tested in two similar, albeit not identical, spawning experiments. Similarly, fertilization replicates used oocytes from individual animals independently tested, but the animals had been acclimated together in group tanks in two nonidentical experiments. Since the lack of identical replicates of acclimation conditions prevents us from applying inferential statistics validly, conclusions are drawn on the basis of the consistency of the findings over similar conditions, refraining from using inferential statistics when these cannot be validly applied, as recommended by Hurlbert (1984). Accordingly, some statements regarding whether a response is "higher" or "lower" are made based on consistency or direction of the data, unaccompanied by a statistical analysis. Tests on non-acclimated animals, however, are true replicates of experimental conditions because all animals came from a common holding tank, and each animal was independently exposed in its own vial to a given temperature/salinity condition. For fertilization, the percentage of fertilized oocytes from several females was analyzed by one-way ANOVA. For spawning, analyses for dichotomous data (spawning *vs.* no spawning of individually tested animals; Fisher's exact test) were used to determine if the occurrence of spawning was randomly distributed (null hypothesis). Extensive experience with testing spawning in zebra mussels in this laboratory in a large number of independent experiments over several years (Ram *et al.*, 1993; Fong *et al.*, 1993, 1994) has indicated that under standard conditions (AW, 20–25°C, during June to mid-August) the percentage of animals spawning in response to 10^{-3} *M* 5-HT ranged between 60% and 100%. Furthermore, when 10–15 animals were tested in independent replicates of each experimental condition, percentage differences in spawning of >30% between treatments were usually statistically significant.

Results

Acclimation and spawning experiments

Results of the first acclimation experiment, including data from non-acclimated animals tested in the same series, are illustrated in Figure 2. The percentage of animals spawning was as high as 90% in AW at 20°C. Without acclimation, spawning in 7.0 ppt occurred in <10% of the animals tested, significantly lower than in AW at all temperatures (Fisher's exact test, for 12°C, $P < 0.006$; for 20°C, $P < 0.00007$; for 27°C, $P < 0.04$, Fig. 2A). Intermediate salinity conditions (1.75 ppt, 3.5 ppt) did not differ markedly from spawning tested in AW at all temperatures, although there is a clear tendency, occurring both with and without acclimation, for spawning to occur in a higher percentage of animals at 20°C than at either 12° or 27°C.

With acclimation, spawning rates in the various salinities and temperatures tended to maintain the same relative positions, with the most notable change being a rise to more than 80% in spawning rates tested in 7.0 ppt after 13 days of acclimation. With 1-day acclimation, 7.0 ppt was still inhibitory to spawning (Fig. 2B). After acclimation for 2 days, spawning rates were higher in all conditions except for 7.0 ppt at 27°C. These increases in percent spawning from 1 day to 2 days of acclimation were particularly notable in 7.0 ppt at both 12°C and 20°C (compare 7.0 ppt in Fig. 2B to Fig. 2C). By the 6th day of acclimation, mussels in all conditions spawned at a high percentage, and this continued to the 13th day (Fig. 2D, E).

A repeat of the acclimation experiment yielded similar results. Without acclimation and with a 1-day acclimation, 7.0 ppt was inhibitory to spawning (Figs. 3A, B). As in the first experiment, where differences in spawning as a function of temperature were present, higher spawning rates were observed at 20°C than at either 12°C or 27°C. By the 4th day of acclimation, mussels in all groups spawned at a high percentage (>70%, Fig. 3C) at all temperatures. Mussels tested on the 15th day had been ac-

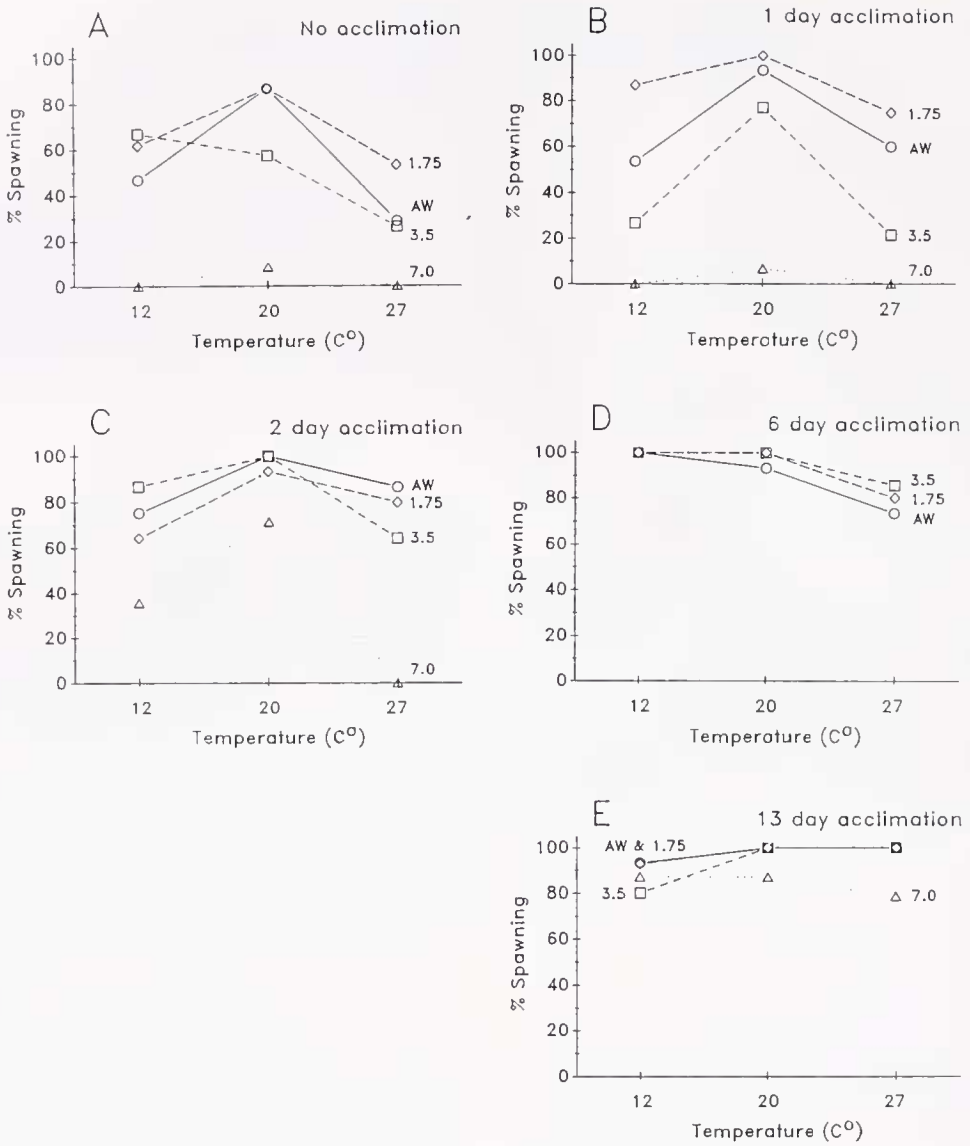

Figure 2. Effect of salinity, temperature, and duration of acclimation on serotonin (10^{-3} *M*)-induced spawning. Zebra mussels were acclimated in aquarium water (AW), 1.75, 3.5, and 7.0 ppt at 12°, 20°, and 27°C and tested for spawning after (A) no acclimation to any salinity/temperature condition (previously maintained in AW, 12°C); (B) 1-day acclimation; (C) 2-day acclimation; (D) 6-day acclimation; and (E) 13-day acclimation. In the 13-day acclimation experiment, mussels tested for spawning in 7.0 ppt were acclimated for 12 days in 3.5 ppt and then transferred to 7.0 ppt for 1 day before testing. Each point represents the percentage of animals spawning out of 12–15 animals tested for each salinity/temperature/duration condition.

climated to a gradual increase in salinity, and all groups spawned at frequencies of 80% or higher (Fig. 3D).

The motility of sperm from non-acclimated mussels was affected by salinity. Sperm taken from aquarium water and transferred directly to either 3.5 or 7.0 ppt exhibited either greatly reduced motility or no movement at all. Sperm transferred to 1.75 ppt had noticeably reduced motility. Sperm spawned from mussels that had been acclimated to higher salinities for 1 to 2 days also showed reduced motility or were completely immotile in all series. By the 6th day of acclimation, however, no obvious reduction in sperm motility was observed at any salinity.

Fertilization experiments

Although 1.75 ppt had no acute or long-term effect on fertilization, higher salinities had inhibitory effects that could be at least partially reversed by acclimation (Fig.

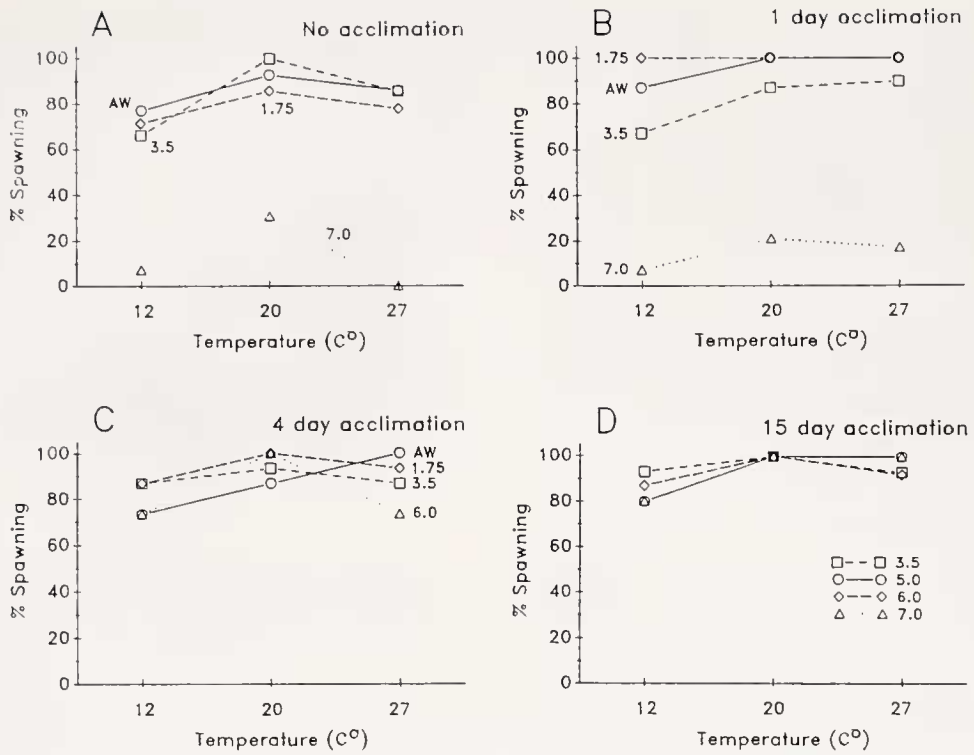

Figure 3. Results of a second experiment on effects of salinity, temperature, and duration of acclimation on serotonin (10^{-3} *M*)-induced spawning. Zebra mussels were acclimated in aquarium water (AW) and various salinities at 12°, 20°, and 27°C and tested for spawning after (A) no acclimation to any salinity/temperature condition (previously maintained in AW, 12°C; (B) 1-day acclimation; (C) 4-day acclimation; and (D) 15-day acclimation. Mussels tested for spawning in 6.0 ppt after 4 days acclimation were acclimated for 3 days in 3.5 ppt and then transferred to 6.0 ppt for 1 day before testing. In the 15-day acclimation tests, all mussels were acclimated gradually to increasing salinities from 1.75 ppt up to their final test salinity as described in the text. All other animals in the experiments were acclimated in their indicated salinities (AW, 1.75, 3.5, and 7.0 ppt) and temperatures (12°, 20°, and 27°C) throughout the acclimation period. Each point represents the percentage of animals spawning out of 10–15 animals tested for each salinity/temperature/duration condition.

4). For non-acclimated mussels, one-way ANOVA of the effect of salinity on log-transformed data of percent fertilization gave $F_{3,12} = 74.2$, $P < 0.0001$ in experiment 1 and $F_{3,8} = 361.8$, $P < 0.0001$ in experiment 2. The percentage of oocytes fertilized in both 3.5 ppt and 7.0 ppt was significantly lower than in both AW and 1.75 ppt (Fisher's LSD, $P < 0.05$ for all four comparisons in both experiments). For acclimated mussels, 7.0 ppt was still inhibitory to fertilization after a 4-day acclimation; however, the point to be noted is that the fertilization rate in 3.5 ppt now overlapped in range with the fertilization rate in AW. The fertilization rate at 3.5 ppt rose from 1.4% ± 0.5% (mean ± SE) in non-acclimated mussels to 37.6% ± 13.6% in 4-day-acclimated mussels. Similarly, after 21 days acclimation, fertilization rates in 3.5 ppt increased to 57% ± 35%, compared to 0% in non-acclimated controls.

During the course of the above fertilization experiments, we noticed that oocytes often ruptured at higher salinities. These observations were quantified in the latter of the two experiments (21-day acclimation and its non-acclimated control, Fig. 5). There was a significant increase in rupturing with elevated salinity in both acclimated and non-acclimated mussels, with and without sperm (one-way ANOVAs: for acclimated mussels without sperm, $F_{2,13} = 61.1$, $P = 0.0001$; with sperm, $F_{2,13} = 5.46$, $P = 0.001$; for non-acclimated mussels without sperm, $F_{3,8} = 6.2$, $P = 0.01$; with sperm, $F_{3,7} = 8.9$, $P = 0.008$). The decrease in oocyte rupturing that occurred in the presence of sperm was apparently due to a protective effect of fertilization. Thus, in 1.75 ppt, in which fertilization occurs at a high rate (Fig. 4), addition of sperm significantly reduced the percentage of oocytes rupturing from >80% to <20% ($P < 0.05$). The effect of sperm addition on the percentage of ruptured oocytes in non-acclimated mussels was not apparent in 3.5 and 7.0 ppt because almost no fertilization occurred at these two salinities. After 21 days of acclimation, in which fertilization occurs at a higher

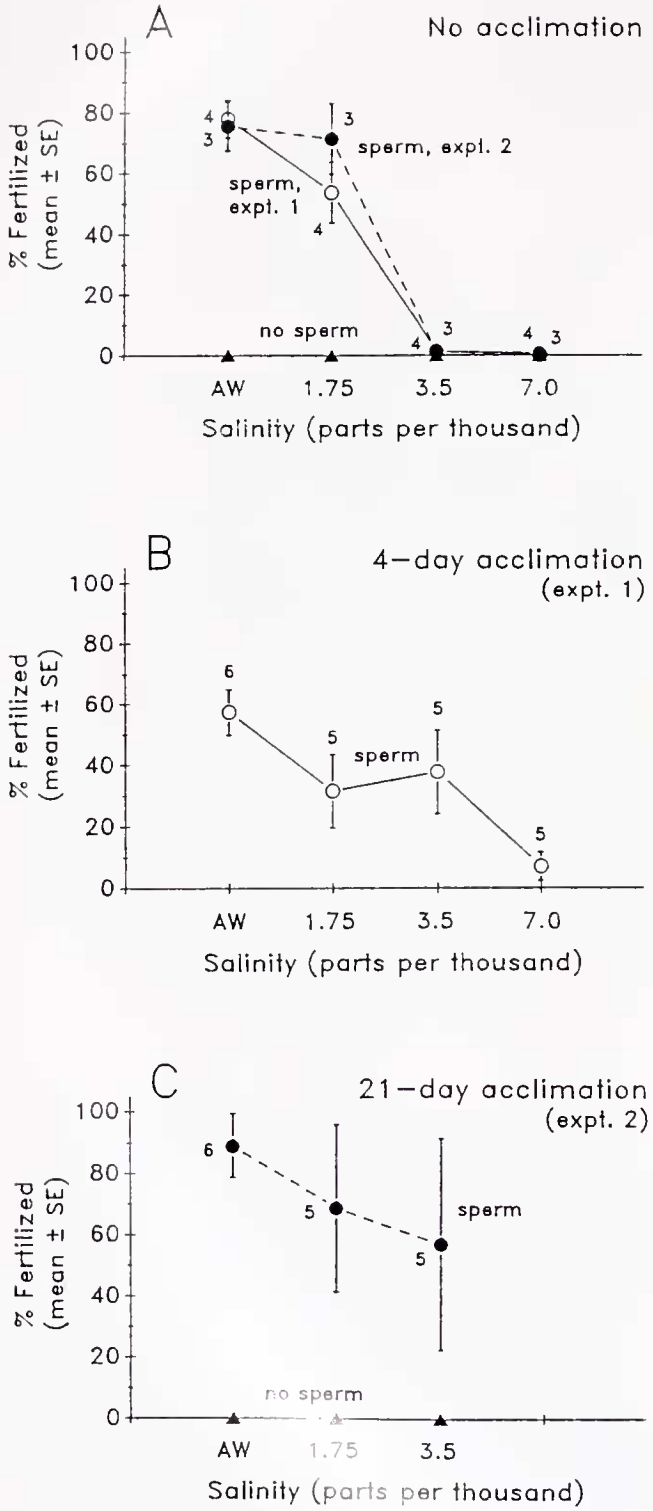

Figure 4. Effect of salinity on fertilization. Percentage of oocytes fertilized when exposed to sperm in aquarium water (AW) and salinities of 1.75, 3.5, or 7.0 ppt after (A) no prior acclimation of source animals (data from two separate experiments); (B) 4-day acclimation of source animals to various salinities at 20°C (experiment 1); and (C) 21-day acclimation of source animals to various salinities (experiment 2). Experiment 2 included "no sperm" controls with each group, none of which resulted in apparent cleavage and development (the end-point for determining that fertilization had occurred). Points and error bars represent the mean ± SE percent of cleaved oocytes observed within 3.5 hours of the addition of sperm. Sample sizes (numbers of females from which oocytes were obtained and tested in separate wells) are given adjacent to error bars.

rate in 3.5 ppt, a significant protective effect of sperm was apparent in both 1.75 ppt and 3.5 ppt ($P < 0.05$).

Because unfertilized oocytes in AW rarely ruptured, and rupturing in 1.75 ppt occurred in >60% of oocytes whether acclimated or not, we wondered what the lower limit of oocyte sensitivity to increased salinity was. To investigate this question, oocytes freshly spawned in AW were exposed to salinities of AW, 0.175, 0.35, 0.7, 1.0, and 1.75 ppt, without sperm, in a 24-well culture plate and observed at intervals of 1 to 2 h. No rupturing occurred after 1 h; however, some oocytes in 1.75 ppt appeared shrivelled. Within 2 h, oocytes began to rupture and, as illustrated in Figure 6, the percentage of ruptured oocytes increased with increasing salinity (linear regression, $r = 0.66$, $P < 0.001$).

Discussion

This is the first study to examine the effects of salinity directly on the reproductive mechanisms of zebra mussels. Previous studies focused on the viability of larvae (Setzler-Hamilton and Wright, 1994) and adults (*e.g.*, Mackie and Kilgour, 1992), and on biogeographic data relating distribution to salt concentrations (Walton, 1992; Strayer and Smith, 1993). The present study has demonstrated that although acute exposure of zebra mussels or their gametes to higher saline conditions is detrimental to spawning, fertilization, sperm motility, and oocyte integrity, acclimation of mussels to elevated saline levels over a period of days results in significant improvement in these indicators of reproductive function.

The improvement in reproductive function with acclimation was particularly striking at 3.5 ppt, the intermediate level of salinity tested. In response to 5-HT, non-acclimated zebra mussels can spawn gametes in salinities from 1.75 to 3.5 ppt, but only rarely in 7.0 ppt (Figs. 2 and 3). Although gametes obtained under these acute conditions can be consistently fertilized at 1.75 ppt, fertilization in 3.5 ppt, without prior acclimation, was practically nil (Fig. 4). Acclimation of animals in 3.5 ppt resulted in increased fertilization rates when tested in 3.5 ppt. Moreover, after acclimation of animals at 3.5 ppt for 4 or more days, spawning could be elicited by 5-HT in salinities as high as 7.0 ppt (Fig. 2 and subsequent experiments). Nevertheless, fertilization rates of oocytes obtained in 7.0 ppt after accli-

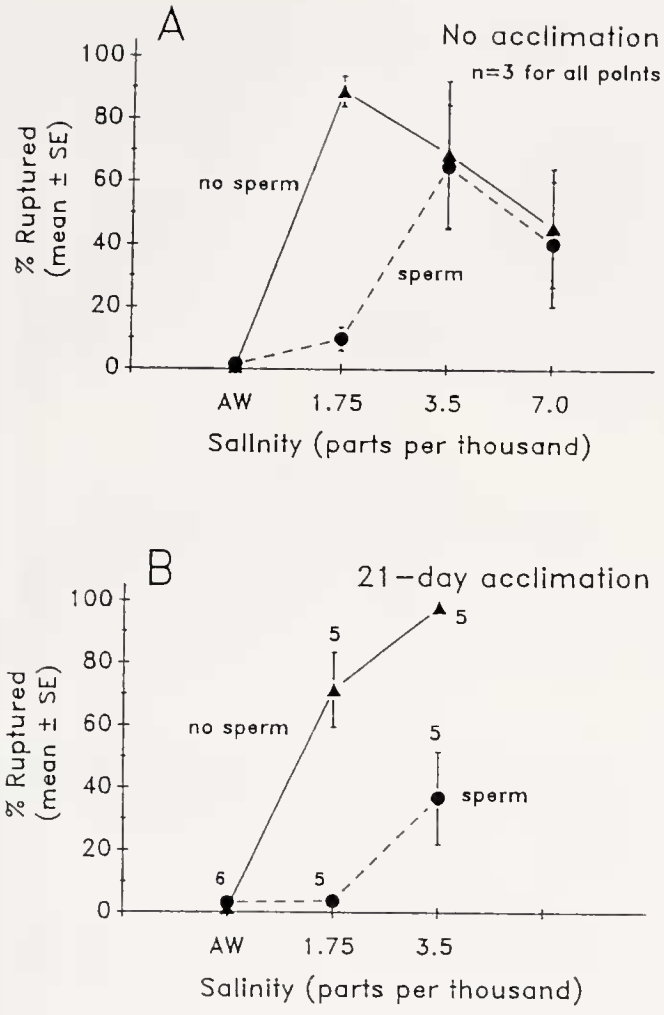

Figure 5. Effect of salinity on oocyte integrity. Percentage of oocytes ruptured was determined within 3.5 h of spawning, for oocytes tested with and without sperm present in aquarium water (AW), and various salinities. (A) Oocytes were from animals not previously acclimated and were tested in AW and salinities of 1.75, 3.5, and 7.0 ppt, $n = 3$ females per group. (B) Oocytes were from animals previously acclimated 21 days at 20°C to AW and salinities of 1.75 and 3.5 ppt and were tested in the same salinities to which they had been acclimated. Sample sizes (number of females from which oocytes were obtained and tested in separate wells) are given adjacent to error bars. Points and error bars represent the mean ± SE percent of oocytes ruptured.

mation remained depressed (Fig. 4). Thus the reproductive function of zebra mussels acclimated for several days to salinities as high as 3.5 ppt recovers to normal levels for several reproductive parameters but, at least within the time-temperature ranges tested, reproductive function at the highest salinity (7.0 ppt) is still significantly below normal.

The acute reduction in fertilization with increasing salinity may be due in part to reduced sperm motility. Sperm from non-acclimated mussels were either immotile or moving slowly in 3.5 and 7.0 ppt, with some reduction in motility at 1.75 ppt. Moreover, mussels acclimated for up to 2 days showed similar immotility in all salinities. Sperm from males acclimated for 6 days showed no reduction in motility, and this may explain the significant increase in fertilization in acclimated animals (albeit tested at 4 and 21 days acclimation) compared to non-acclimated animals tested in the same salinity. It may also explain the trend towards higher fertilization rates between 4-day and 21-day acclimation. In the present study, fertilizations were carried out in about 1 ml of water in 24-well culture plates. This small volume would increase the likelihood of even motility-deficient sperm encountering eggs. In the field, where volumes in which sperm may encounter oocytes are much larger, reduced motility of sperm may reduce fertilization success further than observed here.

Reductions in fertilization success may also be due to osmotic effects on oocytes. Rupture of unfertilized oocytes was evident in salinities as low as 0.175 ppt and increased significantly with salinity even at moderate saline levels. Although oocytes generally took 1 to 2 h to rupture, shrinkage in saline solutions was present (the exact timing and dimensions of such physical changes were not recorded) before rupture. Thus, increased saline levels may reduce the likelihood of fertilization by inducing pathological changes in oocytes between the time that they are exposed to the saline solution and the time that they encounter sperm. Osmotic effects may also explain the reduced motility of non-acclimated sperm. We need to know more about the normal longevity of sperm and egg viability and the normal latency between spawning and fertilization in the field before we can evaluate the importance of salinity-induced oocyte rupture on reproductive success.

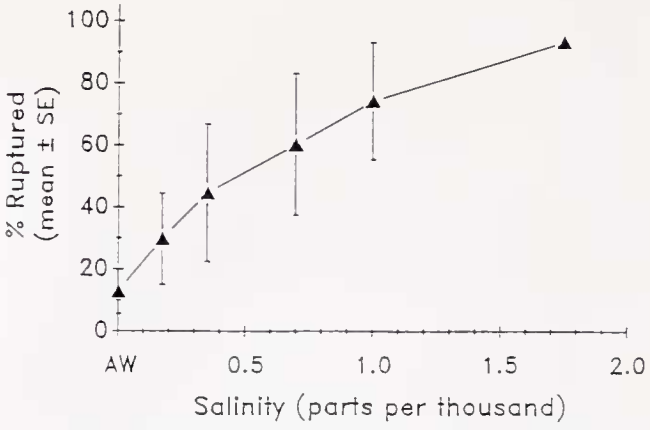

Figure 6. Effect of salinity on integrity of unfertilized oocytes. Female mussels were spawned in aquarium water (AW), and then oocytes were exposed to AW and various salinities up to 1.75 ppt. Oocytes (112 to 144 oocytes from each female under each condition) were observed after 2 h. Points represent the mean ± SE percent of oocytes ruptured for oocytes from 4 females. Linear regression gives $r = 0.66$, with $P < 0.001$.

Fertilization protected oocytes from salinity-induced rupture. Two possible mechanisms for this protection are (1) that secretion of a fertilization envelope provides some ionic/osmotic protection, and (2) that after fertilization, solutes (sugars, proteins, ions) accumulate and provide protection.

Temperature had a modulating effect on spawning in different salinities. Without acclimation, animals were more likely to spawn at 20°C than at either 12°C or 27°C. This was true for three of four salinities in experiment 1 (Fig. 2A) and all four salinities in experiment 2 (Fig. 3A). The higher response at 20°C is in agreement with the results of a previous experiment (Ram *et al.*, 1993), in which the rate of spawning in AW was higher at 20°C that at 12°C or 27°C. These data indicate that sudden rises in temperature into the upper twenties (°C) could inhibit reproductive function in zebra mussels, a suggestion that may explain some of the spawning variations observed in field studies (unpub. data). However, with longer acclimation to higher temperatures, spawning rates under all conditions rose to high levels at all salinities. Thus, despite the somewhat greater inhibitory effects of salinity at high temperatures in non-acclimated mussels, if high temperatures are sustained over long periods, spawning responses can recover to maximal levels within the range of salinity and temperature studied.

Salinity is a limiting factor in the distribution of adult zebra mussels in European brackish waters (Strayer and Smith, 1993). In North America, zebra mussels have spread downstream in the Hudson River estuary as far as West Haverstraw, New York, where salinities reach 4–6 ppt (Walton, 1992). In studies of adult mortality, Mackie and Kilgour (1992) found that the 96-h LC50 for salinity (Instant Ocean) at 19°C was 7.6 ppt for non-acclimated mussels. Acclimated zebra mussels survived longer, but at lower test temperatures. Setzler-Hamilton and Wright (1994) reported that D-hinge larvae of cultured zebra mussels survived at least 48 h at 22°C, but that survival ranged from only 20% at 18°C and 4 ppt to 0.2% at 18°C and 8.0 ppt. The results of our experiments together with these previous observations suggest that the salinity/temperature regime for zebra mussel survival is somewhat less than 7.0 ppt and less than 27°C, and that mussels can reproduce successfully at a salinity of 3.5 ppt. Thus, brackish-water populations of adult zebra mussels could result from both downstream transport of larvae and local recruitment.

This is one of the few investigations into the salinity tolerance of reproductive mechanisms in an invertebrate that abounds in fresh water. Animals living in fresh water must have special adaptations to withstand the osmotic stress of low salinity. Furthermore, if exposure to brackish waters is a possibility, then mechanisms may have evolved for surviving and reproducing in conditions of increasing or varying salinity. In most fresh-water bivalves, fertilization normally occurs within the suprabranchial chamber of the female, from which brooded, parasitic glochidia larvae are released. Zebra mussels represent a rare non-crustacean, freshwater invertebrate with external fertilization and planktotrophic larval development. However, the osmoregulatory mechanisms that allow zebra mussel gametes and larvae to tolerate extremely dilute conditions and to acclimate (as shown here) to brackish-water conditions are unknown. It would be of interest to determine how zebra mussel gametes are protected from osmotic stress in fresh water and what changes take place during acclimation.

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